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(54) **Water insoluble polypeptides**

(57) A pharmaceutical composition designed for sustained and controlled release of drug over an extended period of time comprises a polylactide, a polyglycolide, a copolymer of lactic and glycolic acid or a mixture of these polymers and a water-insoluble peptide which, when placed in an aqueous physiological-type environment releases the peptide in continuous manner for a period of at least one week, and with an initial release for the first twenty-four hours of not more than 30% of the total amount released.

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DESCRIPTION

This invention relates to pharmaceutical compositions of therapeutically active but water-insoluble polypeptides, which provide a continuous, controlled and sustained release of such peptides when placed in a physiological-type environment by means of implant or injections under the skin or into the muscle of animals and humans.

This invention is further characterized by the use of bio-degradable and bio-compatible polymers and copolymers as matrix in which the water-insoluble polypeptides are dispersed or encapsulated.

The need of producing sustained release of peptides for parenteral administration has been recognized for a long time (cf. T.M.S. Chang "Biodegradable Semipermeable Microcapsules containing enzymes, hormones, vaccines and other biologicals" in J. Bioengineering 1, 25 (1976); R. Langer "Controlled Release of Macromolecules" in Chemtech, February 1982, pp 98-105; F.G. Hutchinson and B.J. A. Furr "Biodegradable carriers for the sustained release of polypeptides" in TIBTECH, April 1987 (vol. 5) pp 102-106.

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A number of such formulations, but applied to water soluble polypeptides, have been described in EPS 0052510 "Microencapsulation of water soluble polypeptides", published 27.08.86 and in EPS 0058481 "Continuous release pharmaceutical compositions", published 01.10.86.

The novel, surprising and totally unexpected feature of the present invention resides in the fact that therapeutically useful sustained and controlled release compositions can advantageously be obtained by using essentially water-insoluble peptides, possessing immeasurably low solubility in aqueous solution at room or body temperature and yet providing an effective and controlled release of such peptides when their compositions are administered parenterally in a physiologic, essentially aqueous environment.

It is a novel and surprising consequence of the present invention that polypeptides which are normally water soluble in nature or when prepared by synthesis, can be advantageously rendered water insoluble by forming insoluble addition salts, such as with pamoic acid, tannic acid, stearic acid and other non-toxic water-insoluble acids, prior to their microencapsulation or dispersion in a biodegradable polymeric matrix.

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The use of sparingly soluble or water insoluble derivatives is of course well known, even in the peptide field (cf Schally et al. US Patent 4,010,125 March 1, 1977, column 7, line 25), when slow-release depot dosage forms are needed.

However, when biodegradable polymers such as polylactic acid, polyglycolic acid, polyhydroxybutyric acid, polyortho-esters, polyacetals and the like are used as drug delivery systems, the release of the peptides in a continuous manner has consistently required an appreciable water solubility. Reported experiments have shown that the biodegradation of polymers (such as polylactide and polylactide-co-glycolide for example) leads to water-uptake and generation of aqueous channels or pores from which peptides leak out because they are water soluble.

Our discovery that peptides can be released from matrixes and microcapsules with a highly desirable release pattern when their water solubility is diminished down to practically zero levels is totally surprising and contradicts the teachings of the prior art. In particular we found that the release of certain peptides, such as D-Trp^{6}-LHRH, from polymeric matrixes, is better in terms of uniformity and duration, the more water-insoluble the addition salt of the peptide is.

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"Water-insolubililty" is hereby defined as the amount of peptide which can be measured in solution when the salt is dispersed or stirred for 4 hours in distilled water at temperatures of 40°C or below, such amount beeing 25 mg/l or less (0 to 25 ppm).

It is highly desirable to administer biologically active polypeptides continuously and for a sustained period of time, from one week to several months. It is also highly desirable that the pattern of release be controlled, so as to avoid uneven releases of the peptide at the beginning, in the middle or at the end of the therapeutic cycle. It has been often found that peptides are released from biodegradable matrixes in bursts (also called burst effects), either at the beginning of the cycle or at the end, when the polymeric matrix is eroded through hydrolysis.

An important feature of the present invention is a control of the release pattern, and in general a decrease of the initial burst effect. The water insoluble peptide is released to a lesser extent that its water soluble derivatives, thus affording a more prolonged release time and the avoidance of overdosing the patient. By transforming a normally water soluble peptide into an insoluble one, we are able to limit the initial burst effect (i.e. the amount of peptide released in the first 24 hours) to less than 30% of the total dose.

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Example I

Fifty grams of a copolymer of D,L-lactide and glycolide with a 50/50 molar ratio of D,L-lactide to glycolide and an average molecular weight of 50,000 is dissolved in 950 grams of methylene chloride.

The solution is passed through a millipore filter to remove any particulate matter and pyrogens. To this solution, one gram of D-Trp^6 LHRH pamoate is added and dispersed with a high shear mixer.

The resulting mixture is placed in a rotating evaporator and the majority of the methylene chloride is removed under vacuum. The resulting thick dispersion is poured onto a glass plate and spread with an adjustable blade set at 0.7 mm.

After air drying the resulting film is vacuum desiccated for 48 hours and then extruded through a 0.8 mm orifice at 70°C under pressure. The resulting rods are ground cryogenically at -40°C.

The resulting granular material is sieved through a 180 micrometer screen and the undersize fraction is collected and sterilized by exposure to gamma radiation between 2.5 and 2.8 Mrad.

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Example II

The same procedure as in example I is followed by substituting D-Trp^6-LHRH pamoate with D-Trp^6-LHRH stearate salt.

Example III

The same procedure as in example I is followed with the pamoate salt of D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2 as the water insoluble peptide.

Example IV

The procedure of example I is applied to one of following water-insoluble pamoate salts:

D-Nal(2)^6 LHRH pamoate

D-Ser(O-tBu)^6-des Gly^{10}-Azgly^{10}-LHRH pamoate

D-Ser(But)^6 LHRH(1-9) ethylamide pamoate

D-Leu^6-des Gly^{10}-LHRH ethylamide pamoate

Example V

The procedure of examples I to IV is followed with D,L lactide-co-glycolide polymers in which the molar ratio was 67% D,L lactide 33% glycolide, 75% D,L lactide 25% glycolide or 100% D,L lactide.

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Example VI

The procedure of examples I to V is followed with the water-insoluble pamoate, tannate or stearate salts of one of the following peptides: oxytocin, vasopressin, ACTH, calcitonin, epidermal growth factor, prolactin, inhibin, interferon, LHRH, somatostatin, insulin, glucagon, atrial natriuretic factor, endorphin, a renin inhibitor, GHRH, peptide-T, or synthetic analogues and modifications thereof.

Release pattern in animals(rats)

A typical release pattern of an implanted formulation of D-Trp^6-LHRH pamoate in rats is the following: ng/ml of radio-assayed D-Trp^6-LHRH in plasma (mean of six rats): (t_0) 0.04, (1 hr) 7.74, (6 hrs) 0.80, (day 2) 0.85, (day 4) 0.77, (day 7) 0.25, (day 11) 0.12, (day 14) 0.11, (day 18) 0.11, (day 21) 0.14, (day 25) 0.18.

The preceding examples are not limitative to the described water-insoluble peptides or to the biodegradable polymers used, as it is apparent to a person skilled-in-the-art.

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Claims

1) A pharmaceutical composition designed for sustained and controlled release of drug over an extended period of time comprising a polylactide, a copolymer of lactic and glycolic acid, a mixture of such polymers and a water-insoluble peptide which, when placed in an aqueous physiological-type environment releases the peptide in continuous manner for a period of at least one week, and with an initial release for the first twenty-four hours of not more than 30% of the total amount released.

2) A pharmaceutical composition as claimed in claim I in which the water-insoluble peptide is a pharmaceutically acceptable salt of LHRH or a synthetically prepared analogue thereof.

3) A pharmaceutical composition as claimed in claims I,2 in which the pharmaceutically acceptable salt is selected from the group of pamoate, tannate and stearate salt.

4) A pharmaceutical composition as claimed in claim I in which the water-insoluble peptide is a pharmaceutically acceptable salt of oxytocin, vasopressin, ACTH, calcitonin, epidermal growth factor, prolactin, inhibin, interferon, somatostatin, insulin, glucagon, atrial natriure-

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tic factor, endorphin, a renin inhibitor, growth hormone releasing hormone, peptide T and synthetic analogues and modifications thereof.

5) A pharmaceutical composition as claimed in claims I, 2, 3 in which the water-insoluble peptide is the pamoate salt of D-Trp^6-LHRH.

6) A pharmaceutical composition as claimed in claims 1, 4 in which the water-insoluble peptide is the pamoate salt of D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2.

7) A pharmaceutical composition as claimed in claims 1 to 6 in form of injectable particles ranging in size from 1 to 500 µm.

8) A pharmaceutical composition as claimed in claims 1 to 6 in a solid shape sterilized by gamma radiation and suitable for subcutaneous implant.

9) A pharmaceutical composition as claimed in claims 1 to 7 sterilized with gamma radiation and suspended in a pharmaceutically acceptable carrier suitable for parenteral administration.

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10) A process for preparing a composition as claimed in claims 1 to 6 comprising dispersing a water-insoluble peptide salt into a solution of polylactide, polyglycolide, a copolymer of lactic and glycolic acids or a mixture of such polymers, drying off the solvent and shaping the resulting mixture into solid particles suitable for parenteral injection or subcutaneous implant.

11) A process for preparing a composition as claimed in claims 1 to 7 comprising dispersing a water-insoluble peptide salt into a solution of polylactide, polyglycolide, a copolymer of lactic and glycolic acids or a mixture of such polymers, adding a coacervation agent and pouring the resulting microcapsules in a pharmaceutically acceptable hardening liquid and collecting the microcapsules from this suspension.

Amendments to the claims have been filed as follows

1. A pharmaceutical composition comprising a polymer selected from polyactides, copolymers of lactic and glycolic acid, and mixtures thereof, and a water-insoluble peptide, the composition having the characteristic that, when placed in an aqueous physiological-type environment, the peptide is released in continuous manner for a period of at least one week, but not more than 30% of the total amount of peptide is released within the initial 24 hours.

2. A composition as claimed in claim 1, in which the peptide is a pharmaceutically-acceptable salt of LHRH or a synthetic analogue thereof.

3. A composition as claimed in claim 2, in which the salt is the pamoate, tannate or stearate salt.

4. A composition as claimed in claim 1, in which the peptide is a pharmaceutically-acceptable salt of oxytocin, vasopressin, ACTH, calcitonin, epidermal growth factor, prolactin, inhibin, interferon, somatostatin, insulin, glucagon, atrial natriuretic factor, endorphin, a renin inhibitor, growth hormone-releasing hormone or peptide T, or a synthetic analogue or modification thereof.

5. A composition as claimed in claim 1, in which the peptide is the pamoate salt of D-Trp^{6}-LHRH.

6. A composition as claimed in claim 1, in which the peptide is the pamoate salt of D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2.

7. A composition as claimed in any of claims 1 to 6, in the form of injectable particles ranging in size from 1 to 500 µm.

8. A composition as claimed in any of claims 1 to 6, in a solid shape sterilised by gamma radiation, suitable for subcutaneous implantation.

9. A composition as claimed in any of claims 1 to 7, sterilised with gamma radiation, in which the peptide is suspended in a sterile pharmaceutically-acceptable carrier, suitable for parenteral administration.

10. A composition as claimed in any preceding claim, in which not more than 25 mg of the peptide is soluble in 1 litre of distilled water when dispersed or stirred therein for 4 hours at 40°C or below.

11. A composition as claimed in claim 1, substantially as described in any of the Examples.

12. A process for preparing a composition as claimed in any of claims 1 to 7, comprising dispersing a water-insoluble peptide salt in a solution of polylactide, polyglycolide, a copolymer of lactic and glycolic acids or a mixture of such polymers, drying off the solvent, and shaping the resulting mixture into solid particles suitable for parenteral injection or subcutaneous implantation.

13. A process for preparing a composition as claimed in any of claims 1 to 7, comprising dispersing a water-insoluble peptide salt in a solution of polylactide, polyglycolide, a copolymer of lactic and glycolic acids or a mixture of such polymers, adding a coacervation agent and pouring the resulting microcapsules into a pharmaceutically-acceptable hardening liquid, and recovering the microcapsules therefrom.